

AMENDMENTS TO THE SPECIFICATION

Please amend the paragraph beginning at page 21, line 1, as follows,

Following this mixing, the samples were centrifuged at 5000 x g for 4 minutes to pellet the solids. After centrifugation, the supernatant was discarded. This procedure was repeated 3 times with low salt buffer, with the supernatant discarded after each centrifugation. Then the washing procedure was repeated 3 times with high salt buffer (50 mM potassium phosphate buffer pH 7.02, 1 M NaCl), the supernatant discarded as before. The resulting washed solids representing for the most part plant cell-wall fragments and insoluble starch. The washed solids were equilibrated with 3 times washing with low salt buffer as described above, to obtain same conditions that favor affinity binding of CBM9-2 to cellulosic matrix in affinity chromatography. A representative sample of the solids before biomass interaction study was taken for SDS-PAGE analysis (lane 3). 10 µl of bacterially produced CBM9-2 purified on cellulose (AvicelTM AVICELTM)-affinity column (O.D. @280 nm 0,394), were taken for later SDS-PAGE (lane 2). The purified CBM9-2 had previously been subjected to repeated (4X) dilution and concentration in a ultrafiltration module as a proven method for desorbing any bound glucose from the CBM9-2, so as to regain the cellulose binding affinity characteristic of the protein.

Please amend the paragraph beginning at page 22, line 14, as follows,

The next day the spiked supernatant (100 ml) containing CBM9-2 was fed to a Streamline 25 (Amersham Biotech) chromatography column containing cellulose (AvicelTM AVICELTM). The feed application was done at flowrate 184 cm/h, in expanded bed mode, followed by a washing step with 5 column volumes high salt buffer (1 M NaCl in 50 mM KPO₄, pH 7.02), followed by 5 column volumes of low salt buffer (50 mM KPO₄, pH 7.02). The expanded column bed was allowed to sediment (sedimented bed height =20 cm) and elution was performed at 92 cm/h with 300 ml of elution buffer: 1 M glucose in 50 mM KPO₄, pH 7.02). The elution conditions resulted in a small peak containing the CBM9-2 protein (see FIG. 3).

Please amend the paragraph beginning at page 22, line 23, as follows,

This showed that using the procedure described hereinabove that firstly; CBM9-2 remains in solution unattached to cell-wall fragments and other poorly defined solids from milled barley seed, secondly; it is possible to use polysaccharidic affinity chromatography as described by this invention to capture CBM 9-2 from milled barley-seed extract, thirdly; this can be done by using well defined pharmaceutical grade cellulose (Avicel TM AVICELTM) as a matrix, and fourthly; the affinity chromatography step can be done in expanded bed mode as described by the invention, fifthly; the CBM9-2 purified from barley seed-extract can be eluted of the matrix under gentle conditions avoiding any denaturing steps, as described by this invention. The very same conditions and procedure as described hereinabove, can be applied to purify CBM9-2-fusion proteins from transgenic milled seed. Suitable methods of that kind are described by applicant's copending application "A Non-denaturing process to purify recombinant proteins from plants" filed simultaneously with this application and incorporated herein in full by reference.

Please amend the paragraph beginning on page 23, line 18, as follows,

The extract mixture was spun down in a centrifuge at 6000 rpm for 10 minutes. The supernatant (extract) from individual seeds was collected and applied onto a microwell filterplate (MSHVN45, MilliporeTM MILLIPORETM) packed with cellulose (AvicelTM AVICELTM). The extracts were added to the cellulose in respective wells with mixing every 3 minutes for 15 minutes at room temperature. After 15 minutes vacuum is applied to microwell plate with the aid of a vacuum manifold (Multiscreen resist vacuum manifold - MAVM0960R, MilliporeTM MILLIPORETM) in order to drain the liquid from the cellulose (flow through) in each well. The cellulose is exposed to washing steps, as described in the method described hereinabove: 5 column (i.e. cellulose filled wells) volumes high salt buffer (1 M NaCl in 50 mM KPO₄, pH 7.02), followed by 5 column volumes of low salt buffer (50 mM KPO₄, pH 7.02). Elution was performed with 250 µl of elution buffer (1 M glucose in 50 mM KPO₄, pH 7.02). The eluate was collected by applying vacuum onto the wells of a fresh microwell plate. The eluate was subjected

to a highly specific ELISA CBM9-2 analysis, that is based on specific polyclonal antibodies raised against CBM9-2.